Topic 13 – Myocardial hypoxia, reperfusion, stroke – B

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0350

Serum level of IL-17A and infarct size in patients with acute ST-elevated myocardial infarction

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Background: Myocardial infarction (MI) is one of the main causes of death in the world. Infarct size (IS) is associated with heart failure and mortality following MI. Early reperfusion is currently the most effective treatment to reduce IS resulting from MI. Although reperfusion reduces infarct size, it can also lead to reperfusion injuries. Recently, it was shown that interleukin-17A (IL-17A) is involved in the pathophysiology of reperfusion injuries. However, the correlation between IS and the IL-17A level in humans is unknown. Our aim was to evaluate whether the IL-17A serum level and the IL-17A active fraction was correlated with IS in humans.

Methods: We selected 101 patients who presented with a ST-elevated Myocardial Infarction (STEMI) and 10 healthy controls. For each patient blood samples at admission (H0) and 4 hours after admission (H4) were collected. IL-17A serum levels were assessed using ELISA and the active fraction was assessed with a functional test. IS was determined by peak troponin level and peak CK level for every patient and by cardiac magnetic resonance imaging (CMR) for 20 patients.

Results: The IL-17A serum level was significantly increased in STEMI patients compared to the healthy controls, showing a median value of 0.9 pg/mL Interquartile Range IQR [0.0-3.2] at H0 and 1.0 pg/mL IQR [0.2-2.8] at H4 versus 0.2 pg/mL IQR [0.0-0.7] for healthy controls. The serum level of IL-17A did not correlate with IS (r=–0.02475, p=0.80 at H0 and r=0.04425, p=0.66 at H4 for peak troponin level; r=–0.03390, p=0.74 at H0 and r=0.02276, p=0.82 at H4 for peak CK level and r=–0.2593, p=0.28 and r= –0.2884, p=0.23 for CMR). As for the serum IL-17A level, no correlation was found between the active fraction of IL-17A and IS.

Conclusion: Serum IL-17A level is significantly increased in patients at the early phase of acute MI compared to healthy controls. However, the level of IL-17A in the acute phase of MI does not correlate with IS.

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Exercise training protects the heart against ischemia reperfusion in a mice model of diet-induced metabolic syndrome: no implication of the classic β3 adrenergic receptors – eNOS pathway

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Metabolic syndrome is associated with a higher cardiac vulnerability to ischemia-reperfusion (IR). Regular exercise is recognized to protect the heart. This has been recently attributed to β3-adrenergic receptors (β3-AR) stimulation and subsequent increase in endothelial nitric oxide synthase (eNOS) activation. However, the role of this pathway in exercise-induced cardioprotection in animals with metabolic syndrome is unknown. We thus evaluated the role of the β3-AR/eNOS pathway in exercise-induced cardioprotection in a mouse model of metabolic syndrome. C57Bl6 mice were fed with high fat and sucrose diet (HFS) for 12 weeks and some had treadmill-exercise with a moderate intensity the last 4 weeks (HFS-Ex). First, HFS hearts were more sensitive to IR, which was prevented by exercise. Even though exercise protects the HFS heart, this was not associated with increased activation state of eNOS (level of eNOS-Pser1177 and the dimer/monomer ratio). Consequently, no increase in NO metabolites storage was observed after exercise in HFS hearts. This result may be explained by the loss of the β3-AR-eNOS pathway in HFS hearts. Indeed, the use of BRL37344, an agonist of β3-AR, increased eNOS-Pser1177 and protected the heart of Ctrl mice, whereas it had no effect in HFS hearts. Finally, considering that exercise-induced cardioprotection is also classically associated with increased antioxidant status, we next evaluated ROS production during early reperfusion and the subsequent activation of the apoptosis end-effector caspase 3. At early reperfusion we found that HFS increased ROS and caspase 3 activation. This phenomenon was blunted in HFS-Ex. Finally, a treatment with LPBNAH, a more amphiphilic nitrone antioxidant derived from PBN normalized HFS heart vulnerability to IR. To conclude these results showed that exercise-induced cardioprotection in HFS hearts is independent of the classical β3-AR-eNOS pathway, but involved oxidative stress during IR.

0391

Molecular mechanisms of ANGPTL4-induced regulation of vascular integrity

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Cardiovascular ischemic injuries are associated with vascular damage induced by loss of endothelial junction integrity. Deciphering the mechanisms involved in the regulation of vascular integrity is of major interest in order to develop relevant therapeutic cardioprotective approaches to achieve tissue protection. Our team identified angiopoietin-like 4 (ANGPTL4) as a hypoxia-induced target and a key regulator of vascular integrity by protecting inter-endothelial cell junctions. However ANGPTL4 receptors and downstream signaling pathways which mediate vasoprotective effect remain poorly investigated. Using both *in vitro* binding (Surface Plasmon Resonance, SPR) and functional *in vivo* assays, we show that ANGPTL4 binds αvβ3 integrin and that this interaction is necessary to mediate vasoprotective effects. In addition, ANGPTL4 induces phosphorylation of Tyr^{773} of β_3 integrin subunit and reorganization of focal adhesions in endothelial cells. Mechanistically, binding of ANGPTL4 to αvβ3 leads to Src recruitment and its sequestration away from VEGFR2 combined to a diminished Src signaling downstream VEGFR2, thereby inducing stabilization of both VEGFR2/VE-cadherin and VEGFR2/αvβ3 complexes. Thus, ANGPTL4 strengthens maturation of adherens junctions, characterized by a transition from a zipper to linear organization of VE-cadherin organization.

Altogether, our results identify a novel mechanism by which ANGPTL4 counteracts hypoxia-driven vascular permeability through αvβ3 binding, modulation of VEGFR2/Src kinase signaling and endothelial junction stabilization.

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The increase in myocardial infarct size induced by intermittent hypoxia and HIF-1 activation, is attenuated by endoplasmic reticulum stress inhibition

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Introduction: Obstructive sleep apnea (OSA) is a highly prevalent disease characterized by repetitive upper airway collapse during sleep leading to chronic intermittent hypoxia (IH). It has been shown in patients that OSA contributes to myocardial infarct expansion. The lack of knowledge about the mechanisms involved in OSA-associated cardiovascular complications had

limited the development of specific treatment whereas the gold standard treatment is little tolerated. In the context of cardiomyocytes death or life, this study purposes to investigate the role of endoplasmic reticulum (ER) stress and hypoxia inducible factor-1 (HIF-1) in myocardial susceptibility to ischemia-reperfusion (I/R) induced by chronic IH.

Methods: C57Bl6J, HIF-1$\alpha^{+/-}$ and their control mice were exposed to 14 days of IH (21–5% FiO2, 60s cycle, 8h/day). Then, mice were submitted to an in vivo ischemia-reperfusion to assess infarct size (IS, in % relative to area at risk) or hearts were removed to assess ER stress markers and HIF-1 activity using Western-blot and ELISA. In additional groups, TUDCA (an ER stress inhibitor, 75mg. kg^{-1}) was administered daily during N or IH exposition to assess the role of ER stress in IH-susceptibility to I/R.

Results: Whereas chronic IH induced an increase in infarct size (33.7±9.4 *vs* 61.0±5.6% in N and IH groups, respectively, p<0.05), IH failed to increase infarct size in HIF1$\alpha^{+/-}$ mice (42.4±2.7 *vs* 24.7±3.4 % in HIF1$\alpha^{+/-}$-N and HIF1$\alpha^{+/-}$-IH, respectively). An increase in HIF-1 activity and ER stress markers was also observed in IH-mice. By the way, TUDCA totally abolished the IH-increased in infarct size (49.9±3.0 *vs* 61.0±5.6% in IH-TUDCA, respectively) as well as the IH-increased in HIF-1 activity (1.3±0.04 *vs* 0.14±0.02 fold increase in IH and IH/TUDCA, p<0.0001 *vs* non treated mice).

Conclusion: These results suggest that the "ER stress-HIF-1"axe should be considered in apneic patients as a potential therapeutic target to limit myocardial ischemic damages.

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Therapeutic hypothermia induced by total liquid ventilation reduces cardiac and cerebral production of free radicals after non shockable cardiac arrest

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Introduction: Ultra-fast cooling induced by total liquid ventilation (TLV) has been shown to be potently neuro and cardioprotective after shockable cardiac arrest and/or acute myocardial infarction**.** In this study we examined a possible underlying mechanism of this protection, in particular reduced free radicals production.

Methods and Results: Thirty six rabbits subjected to asphyxia cardiac arrest were divided into three groups: normothermic life support (Control group, n=12) or hypothermia induced by either i.v. cold saline (CONV group; n=12) or by TLV (TLV group, n=12). Using electronic paramagnetic resonance spectroscopy (EPR) and 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine hydrochloride (CMH) as spin probe, we observed a decrease in the production of free radicals in various organs including the heart and the cerebral cortex in TLV and CONV groups as compared to Control, however the effect of TLV was significantly more marked. These results were associated with a significant improvement of the neurological status and an increase in the survival rate as compared to Control and CONV groups.

Conclusion: Therapeutic hypothermia induced by TLV could be a promising approach to improve organ preservation before irreversible alteration by free radicals.

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Cardioprotective effect of a novel snake venom derived natriuretic peptide during myocardial ischemia reperfusion injury

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Introduction: In this study, we aimed at testing the therapeutic potential of a novel Natriuretic Peptide (NPs), identified from Tunisian snake venom, when administered during reperfusion.

Materials and Methods: Langendorff perfused male Wistar rat hearts were subjected to 30min regional coronary artery occlusion followed by 90min reperfusion. Hearts were treated either with brain natriuretic peptide BNP (10nM) or NPs (200nM). In another set of experiments, hearts were pretreated with either isatin (100µM), a natriuretic peptide receptors blocker or 5HD (10µM), a mitochondrial KATP channels blocker. Post ischemic cardiac haemodynamic parameters, using Labt Chart (ADInstruments) and infarct size (IS), using planimetry, were evaluated. Western blotting experiments for studying cGMP and Reperfusion Injury Salvage Kinases pathways were performed. Calcium Retention Capacity (CRC) to evaluate the mitochondrial function was also assessed by oxygraphy.

Results: BNP and NPs significantly decreased the IS by 60% and 62% respectively compared to control non treated hearts (p<0.001). Regarding hemodynamic parameters, both BNP and NPs improved the developed pressure (DP) by 135 % and 152 % respectively (p<0.05). These beneficial effects are abolished after pretreatment by isatin or 5HD. NPs significantly increased the expression of pAKT, pGSK3ß and PKCε when BNP increased pERK1/2 compared to control group. Both BNP and NPs increased the CRC by 124% and 86% respectively compared to control group.

Conclusion: Our results demonstrate that NPs and BNP have cardioprotective effects during acute myocardial ischemia. These effects are mediated, for both drugs, by natriuretic receptors and by the activation of mitochondrial KATP channels. The cardioprotection involves the two PI3K/Akt/ERK and cGMP-PKC signaling pathways which converge to the mitochondria. Administration of NPs may provide a novel therapeutic strategy in acute myocardial isch